Effect of different antihypertensive treatments on Ras, MAPK and Akt activation in hypertension and diabetes

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ABSTRACT

Ras GTPases function as transducers of extracellular signals regulating many cell functions, and they appear to be involved in the development of hypertension. In the present study, we have investigated whether antihypertensive treatment with ARBs (angiotensin II receptor blockers), ACEi (angiotensin-converting enzyme inhibitors) and diuretics induce changes in Ras activation and in some of its effectors [ERK (extracellular-signal-regulated kinase) and Akt] in lymphocytes from patients with hypertension without or with diabetes. ACEi treatment transiently reduced Ras activation in the first month of treatment, but diuretics induced a sustained increase in Ras activation throughout the 3 months of the study. In patients with hypertension and diabetes, ARB, ACEi and diuretic treatment increased Ras activation only during the first week. ACEi treatment increased phospho-ERK expression during the first week and also in the last 2 months of the study; however, diuretic treatment reduced phospho-ERK expression during the last 2 months of the study. In patients with hypertension and diabetes, antihypertensive treatments did not induce changes in phospho-ERK expression in lymphocytes. ACEi treatment reduced phospho-Akt expression in patients with hypertension and diabetes only in the first month of treatment. In conclusion, these findings show that antihypertensive treatments with ACEI, and diuretics to a lesser extent, modify Ras activation and some of its signalling pathways, although in different directions, whereas ARBs do not appear to have any influence on Ras signalling pathways.

Key words: Akt, antihypertensive medication, diabetes, hypertension, mitogen-activated protein kinase (MAPK), Ras GTPase.

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor(s); AngII, angiotensin II; ARB, AngII receptor blocker; AT1 receptor, AngII type 1 receptor; AT2 receptor, AngII type 2 receptor; BMI, body mass index; BP, blood pressure; ECM, extracellular matrix; ENaC, epithelial Na+ channel; ERK, extracellular-signal-regulated kinase; 20-HETE, 20-hydroxyeicosatetraenoic acid; HRP, horseradish peroxidase; IL, interleukin; IMDM, Iscove’s modified Dulbecco’s medium; MAP, mean arterial pressure; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MLB, magnesium lysis buffer; PI3K, phosphoinositide 3-kinase; RBD, Raf/Ras-binding domain; SAP, systolic arterial pressure; TGF-β1, transforming growth factor-β1; VSMC, vascular smooth muscle cell.

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INTRODUCTION

The Ras family of proteins is formed by a group of small GTPases that function as transducers of extracellular signals regulating cell survival, growth and differentiation, and they are well known by their pro-oncogenic effect [1,2]. Ras GTPases appear to be involved in the development of hypertensive processes, as described by several authors. Muthalif et al. [3] indicated that the activation of Ras contributes to the development of AngII (angiotensin II)-dependent hypertension and associated vascular pathology; more recently, Benter et al. [4] suggested that inhibition of Ras-GTPase-mediated signalling can attenuate end-organ damage during severe hypertension and endothelial dysfunction. In patients with hypertension, AngII promotes the synthesis of the arachidonic acid metabolite 20-HETE (20-hydroxyeicosatetraenoic acid), which contracts blood vessels and causes hyperplasia of VSMCs (vascular smooth muscle cells), contributing to an increase in BP (blood pressure) [5]. Moreover, it has been shown that 20-HETE stimulates Ras and MAPK (mitogen-activated protein kinase) activity, and thus mediates VSMC proliferation through this pathway [6]. These authors demonstrated that arachidonic-acid metabolite– (mainly 20-HETE) induced MAPK activation through Ras contributes to the vascular alterations, hypertrophy and hypertension induced by AngII in rats [3]. Okada et al. [7] have shown that AT1 and AT2 receptors (AngII type 1 and 2 receptor respectively) reciprocally modulate pro-inflammatory and pro-fibrotic reactions in lymphocytes. It has been also been demonstrated that the antihypertensive treatment with losartan, an ARB (AngII receptor blocker), reduces DNA synthesis in T-lymphocytes [8], although, in the same year, Petrov et al. [9] showed that losartan had no antiproliferative effects on mononuclear cells obtained from peripheral blood. Other authors have demonstrated that the stimulation of T- [10] and B- [11] lymphocyte antigenic receptors induces Ras activation. Ehrhardt et al. [12] have shown that the stimulation of B- and T-lymphocyte antigenic receptors induces H-, N- and K-Ras activation, whereas the stimulation of these cells with growth factors, such as IL (interleukin)-3 or EGF (epidermal growth factor), activates K-Ras preferentially.

Ras activates several intracellular signalling pathways [1,2,13,14], including the ERK (extracellular-signal-regulated kinase) MAPK pathway which regulates cell growth, differentiation and apoptosis, and ERK activates many genes through the stimulation of different transcription factors. Another signalling pathway stimulated by Ras through PI3K (phosphoinositide 3-kinase) is the Akt (also known as PKB (protein kinase B)) pathway. Akt also regulates the expression of a large group of genes, induces protein synthesis and protects cells from apoptosis, promoting cell survival. One of the functions of Akt is the phosphorylation of eNOS (endothelial NO synthase) [15,16]. Previous studies from our group in myoblasts have shown that TGF-β1 (transforming growth factor-β1) induces ECM (extracellular matrix) synthesis through activation of another MAPK, p38 MAPK [17]; moreover, the induction of tubule-interstitial fibrosis by unilateral urethral obstruction is associated with Ras, ERK and Akt activation [18]. We have shown that, in the absence of H- and N-Ras isoforms, fibroblasts synthesize larger amounts of ECM proteins and have an increase in Akt phosphorylation, suggesting that H- and N-Ras isoforms down-regulate ECM synthesis via PI3K/Akt signalling. The lack of both H- and N-Ras isoforms is accompanied by a smaller proliferative rate in response to TGF-β1, together with a decrease in ERK phosphorylation, suggesting that H- and N-Ras isoforms mediate proliferation through MEK (MAPK/ERK kinase)/ERK activation [19].

In the present study, we have investigated the involvement of Ras in hypertension and diabetes, evaluating changes in Ras activation and in phosphorylation of its effectors ERK1/2 and Akt before and after antihypertensive treatment with ARBs, ACEi (angiotensin-converting enzyme inhibitors) and diuretics. The endothelium is probably one of the most important tissues involved in the development of hypertension, but in view of the fact that it is not possible to perform these studies on renal and vascular tissues in patients with hypertension and diabetes, we have used lymphocytes obtained from peripheral blood of these patients. These cells appear to be a suitable model for analysing these intracellular pathways in the development of hypertension, as altered T-cell activities and changes in T-cell subset ratios have also been reported in hypertension [8], and interstitial infiltration of T-cells plays a role in the pathogenesis of salt-sensitive hypertension [20]. In addition, a very recent study by Crowley et al. [21] in a mouse model of severe hypertension has demonstrated the robust contribution of lymphocyte proliferation to the pathogenesis of AngII-induced end-organ injury. Furthermore, lymphocytes have AT1 and AT2 receptors [7], and the stimulation of T- and B-lymphocyte antigenic receptors induces Ras activation [10–12].

MATERIALS AND METHODS

Materials and reagents

PMSE, Tween 20, BSA, human serum and SDS were purchased from Sigma. Rabbit anti-(mouse Akt1/2), rabbit anti-(rat ERK1), mouse anti-(human phospho-ERK) antibodies were from Santa Cruz Biotechnologies. The rabbit anti-(human phospho-Akt) antibody was purchased from Cell Signaling Technology. MLB (magnesium lysis buffer) was obtained from Upstate. HRP (horseradish peroxidase)-conjugated goat anti-rabbit IgG and goat anti-(mouse IgG), nitrocellulose
membrane and the DC protein assay were from Bio-Rad Laboratories. ECL® chemiluminescence Western blotting system and Hyperfilm X-ray film were obtained from Amersham Biosciences. IMDM (Iscove’s modiﬁed Dulbecco’s medium) was from BioWhittaker Laboratories. Ras GTPase Chemi ELISA kit was from Active Motif. The sterile plastic material used in cell culture was obtained from Nunc. The Fluoroskan Ascent luminometer was from Thermo Scientific. All other reagents were of analytical grade and were obtained from Sigma, Probus and Merck.

Groups of patients, treatments and extraction of blood samples

These studies were performed in patients with essential hypertension without or with diabetes. Subjects enrolled in the study from July 2004 until January 2008 and were from the Hypertension Unit of the University Hospital of Salamanca, Nephrology Service of the Virgen de la Concha Hospital of Zamora, Health Centre of Matilla de los Caños, Salamanca and the Health Centre of La Alamedilla, Salamanca, Spain. Altogether, 41 patients with hypertension without diabetes and 40 patients with hypertension and diabetes complied with the inclusion/exclusion criteria and were asked to participate in the study. Inclusion criteria were newly diagnosed essential arterial hypertension (BP 140/90 mmHg, or 130/80 mmHg in patients with diabetes) without or with (blood glucose > 125 mg/dl) diabetes, mainly Type 2 diabetes mellitus. Exclusion criteria included previous antihypertensive therapy, serious pathologies (other than hypertension and diabetes), renal or hepatic failure, cardiac congestive insufficiency, treatment with corticosteroids, non-steroid anti-inﬂammatory drugs and other potential hypertension-inducing drugs, heavy smoking and alcoholism. Demographic data of the patients in each group are shown in Table 1; all recruited patients were overweight [BMI (body mass index) between 25 and 29.9 kg/m²; Table 1]. The efficacy of the antihypertensive treatments is shown in Table 2. At the beginning of the study, patients with hypertension and diabetes had higher SAP (systolic arterial pressure) and MAP (mean arterial pressure) compared with patients with hypertension without diabetes; MAP, 178.26 ± 70.42 and 158.39 ± 29.07 mmHg in patients with hypertension and diabetes compared with patients with hypertension without diabetes; MAP, 115.67 ± 1.73 mmHg in patients with hypertension and diabetes compared with patients with hypertension without diabetes). Each antihypertensive treatment induced similar reductions in BP, although ACEI induced the largest decrease in patients with

Table 1  Demographic and physical characteristics of patients with hypertension (n = 41) and patients with hypertension and diabetes (n = 40) before antihypertensive therapy

Values are means ± S.E.M. These patients were randomized to treatment with ARBs, ACEi and diuretics and completed the study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ARBs</th>
<th>ACEi</th>
<th>Diuretics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female/male)</td>
<td>7/6</td>
<td>13/6</td>
<td>3/6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.12 ± 8.6</td>
<td>61.89 ± 2.95</td>
<td>61.50 ± 6.62</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>1.67 ± 0.03</td>
<td>1.63 ± 0.02</td>
<td>1.60 ± 0.03</td>
</tr>
<tr>
<td>Height (m)</td>
<td>2.70 ± 1.07</td>
<td>2.90 ± 0.90</td>
<td>2.93 ± 1.03</td>
</tr>
</tbody>
</table>

Table 2  BP characteristics of the patients with hypertension (n = 41) and patients with hypertension and diabetes (n = 40) before (basal) and after (3 months) antihypertensive therapy

Values are means ± S.E.M. DAP, diastolic arterial pressure.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with hypertension receiving</th>
<th>Patients with hypertension and diabetes receiving</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAP (mmHg)</td>
<td>151 ± 5</td>
<td>174 ± 5</td>
</tr>
<tr>
<td>DAP (mmHg)</td>
<td>93 ± 4</td>
<td>93 ± 3</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>112 ± 4</td>
<td>120 ± 3</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>126 ± 5</td>
<td>156 ± 7</td>
</tr>
<tr>
<td>DAP (mmHg)</td>
<td>80 ± 3</td>
<td>85 ± 3</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>95 ± 3</td>
<td>109 ± 4</td>
</tr>
</tbody>
</table>
hypothesis without diabetes (36 mmHg reduction in SAP, and 24 mmHg reduction in MAP) and in patients with hypertension and diabetes (21 mmHg reduction in SAP, and 14 mmHg reduction in MAP).

The experimental protocol performed was in accordance with the Declaration of Helsinki (2000) of the World Medical Association, and also in agreement with the guidelines and approved by the Ethics Committee of the University Hospital of Salamanca, Salamanca, Spain. Each patient included in the study gave his/her consent to participate after full explanation of the purpose and nature of all procedures used.

The groups of patients were as follows: (i) 13 patients with hypertension without diabetes treated with ARBs [600 mg/day eprosartan (\(n = 9\)), 50 mg/day losartan (\(n = 2\)) or 80 mg/day valsartan (\(n = 2\))]; (ii) 19 patients with hypertension without diabetes treated with ACEi [either 20 mg/day enalapril (\(n = 12\)) or 2 mg/day trandolapril (\(n = 7\))]; (iii) nine patients with hypertension without diabetes treated with diuretics [either 2.5 mg/day indapamide (\(n = 7\)) or 12.5 mg/day hydrochlorothiazide (\(n = 2\))]; (iv) 23 patients with hypertension and diabetes treated with ARBs [600 mg/day eprosartan (\(n = 2\)), 50–100 mg/day losartan (\(n = 2\)), 80–160 mg/day valsartan (\(n = 18\)) or 150 mg/day irbesartan (\(n = 1\))]; (v) nine patients with hypertension and diabetes treated with ACEi [20–40 mg/day enalapril (\(n = 4\)), 2 mg/day trandolapril (\(n = 3\)), 20 mg/day lisinopril (\(n = 1\)) or 10 mg/day imidapril (\(n = 1\))]; and (vi) eight patients with hypertension and diabetes treated with diuretics [either 12.5 mg/day hydrochlorothiazide (\(n = 6\)) or 40 mg/day furosemide (\(n = 2\))].

Peripheral blood samples (10 ml) were obtained by venipuncture after 12 h of fasting, before the treatments (basal) and after 1 week and 2 and 3 months of each antihypertensive treatment.

**Extraction of lymphocytes from blood samples and lymphocyte culture**

Lymphocytes can be separated from granulocytes and erythrocytes according to their lower densities, as they will float on top of a density gradient of Ficoll–Hypaque, whereas granulocytes and erythrocytes will traverse this fluid and collect at the bottom of the tube [22]. A portion (10 ml) of whole blood was centrifuged at 160 g for 5 min at room temperature (22°C), and the yellow-coloured supernatant (approx. 1 ml) containing platelets was removed. The same volume of PBS at room temperature was added and mixed. A total of 5 ml of blood was added carefully on top of 4 ml of Ficoll–Hypaque, and the tubes were centrifuged at 400 g for 30 min at room temperature. The interface band (which includes the lymphocytes) was aspirated along with no more than 5 ml of fluid above the pellet, and was transferred to new tubes. The volume was completed with PBS and centrifuged at 400 g for 30 min at room temperature. The lymphocyte pellet was washed twice with PBS and centrifuged again. A fraction of the lymphocytes obtained was cultured in IMDM supplemented with 10 % (v/v) human male AB plasma, penicillin (0.66 mg/ml) and streptomycin sulfate (60 mg/ml) [23].

Monocytes/macrophages comprise 5–20 % of the lymphocytes prepared by density gradient centrifugation. To isolate T- and B-cell populations, we exploited the adherence property of monocytes/macrophages by coating plastic tissue culture vessels with serum (as a source of fibronectin and other components of the ECM). Monocytes/macrophages, but not T- and B-cells, adhered to these surfaces, and we finally isolated the T- and B-lymphocytes which remained in the supernatant.

**Cell lysates**

Lymphocyte extracts were obtained by cell lysis in MLB [25 mmol/l Hepes (pH 7.5), 150 mmol/l NaCl, 1 % Igepal CA-630, 0.25 % sodium deoxycholate and 1 mmol/l EDTA] containing 10 % (v/v) glycerol and proteases and phosphatases inhibitors (1 mmol/l PMSF, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 1 mmol/l sodium vanadate and 25 mmol/l sodium fluoride). Cell extracts were maintained at 4°C for 30 min and then centrifuged at 15 000 g for 20 min at 4°C. Supernatants were collected and the protein content of cell extracts was determined by the Lowry assay (DC protein assay).

**Detection of Ras-GTP by ELISA**

Ras-GTP (the active form of Ras) was detected by the Ras GTPase Chemi ELISA kit, which contains an RBD ( Raf/Ras-binding domain) protein fused to GST (glutathione transferase) and is coated on to a 96-well glutathione-coated plate. The activated Ras contained in cellular extracts specifically binds to Raf–RBD, whereas inactive Ras does not bind. Bound Ras is detected by incubating with a primary antibody that detects H- and K-Ras in human samples. Addition of a secondary antibody conjugated with HRP and a developing solution provides a sensitive chemiluminescent readout, which is quantified by luminescence in a luminometer. The assay was performed according to the manufacturer’s instructions, and luminescence was quantified on a Fluoroskan Ascent luminometer.

**Western blot analysis**

Fresh lymphocyte extracts were used in the detection of Akt1/2, phospho-Akt, ERK1/2 and phospho-ERK. Equal amounts of proteins (20–30 μg) were electrophoresed on 10 % (w/v) (for Akt and phospho-Akt) and 12 % (w/v) (for ERK and phospho-ERK) SDS/polyacrylamide gels and transferred on to nitrocellulose membranes (0.45 μm). Membranes were blocked with blocking buffer [1–5 % (w/v) BSA in 20 mmol/l Tris/HCl, 150 mmol/l NaCl and 0.1 %
Tween 20, pH 7.5–8.0] and incubated with anti-Akt1/2 (1/1000 dilution), anti-ERK1 (1/10000 dilution), anti-(phospho-ERK) (1/2000 dilution) and anti-(phospho-Akt) (1/1000 dilution). After incubation with the corresponding HRP-conjugated secondary antibody (1/10000–1/20000 dilution), membranes were finally incubated with ECL® detection reagent and the developed signals were recorded on X-ray film (Hyperfilm) for densitometric analysis (Scion Image for Windows).

**Statistical methods**

Values are means ± S.E.M. The Kolmogorov–Smirnov test was used to assess the normality of the data distribution. Comparison of means was performed by one-way ANOVA. Statistical differences between groups were assessed by Scheffe’s test. A P value < 0.05 was considered statistically significant.

**RESULTS**

**Effect of antihypertensive treatment on Ras activation**

The amount of activated Ras (Ras bound to GTP) in lymphocyte extracts obtained from patients before and after different antihypertensive treatments was determined. ARB treatment in patients with hypertension without diabetes did not induce any significant change in Ras activation during the 3 months of the study, while ACEi treatment reduced Ras activation after 1 month, but had no effect in the second month of treatment (Figure 1A). In contrast, diuretics induced a sustained increase in Ras activation after 1 week of treatment (Figure 1). In patients with hypertension and diabetes, each antihypertensive treatment increased Ras activation in the first week, but these increases returned to basal after 1 month of treatment (Figure 1B).

**Effect of antihypertensive treatment on ERK activation**

Treatment with ARBs did not induce significant changes in phospho-ERK expression in lymphocytes from patients with hypertension without diabetes, although there was a slight increase in phospho-ERK expression in the last 2 months of the study; ACEi treatment induced a significant increase in phospho-ERK expression in the first week, which returned to basal after 1 month, but was increased again in the last 2 months of the study (Figure 2A). In contrast, diuretic treatment reduced phospho-ERK expression in the last 2 months of the study (Figure 2). In patients with hypertension and diabetes, antihypertensive treatments did not induce significant changes in phospho-ERK expression in lymphocytes, although diuretics and ACEi reduced phospho-ERK expression in the first and third months of treatment respectively (Figure 2B).

**DISCUSSION**

In the present study, we have evaluated the effect of antihypertensive treatments on Ras, ERK1/2 and Akt activation in patients with hypertension without or with diabetes mellitus, preferably Type 2 diabetes, although most patients with this pathology usually take antihypertensive drugs. Therefore we were not able to recruit a larger number of patients, due to the exclusion criteria of previous antihypertensive therapy. Moreover, the inclusion criteria of newly diagnosed essential arterial
hypertension reduced the number of available patients who could be included in our study.

Diabetes is the most frequent cause of chronic renal failure, and one of the main characteristics of this pathology is the appearance of hypertension. Ras proteins play a relevant role in the development of diabetes, as it has been described widely that (i) transgenic mice expressing an H-ras hybrid gene in pancreatic β-cells develop β-cell degeneration and diabetes [24]; (ii) inhibition of signal transduction involving Ras GTPase can prevent the development of diabetes-induced abnormal vascular reactivity in the renal artery [25]; (iii) Ras induction of superoxide activates ERK-dependent fibrosis-stimulatory factor and ECM gene transcription in mesangial cells in diabetes-induced renal injuries [26]; and (iv) H-Ras activation is also important in the activation of the specific signalling events leading to the accelerated retinal capillary cell apoptosis in hyperglycaemic conditions, suggesting the possible use of H-Ras inhibitors to inhibit the pathogenesis of diabetic retinopathy [27,28].

The presence and activity of Ras GTPases have been widely described in lymphocytes. Many members of the Ras family of GTPases, notably Ras and Rap1A, and the Rho family of GTPases, such as Cdc42Hs, Rac1, Rac2 and RhoA, are important components of signal...
transduction pathways used by antigen, cytokine and chemokine receptors to regulate the immune response in lymphocytes [29]. Transduction of activation signals in T-cells involves the redundant participation of the Ras superfamily of GTPases [30]. The first step is T-cell receptor ligation, which leads to the activation of the Ras/MAPK signal transduction pathway [10]. T-cell receptor activation leads to PLCγ (phospholipase Cγ)-mediated translocation of the nucleotide-exchange factor RasGRP1 (guanine-nucleotide-releasing protein 1) to the Golgi apparatus where it activates Ras [31]. Recently, Ruiz et al. [32] have shown that RasGRF2 (guanine-nucleotide-releasing factor 2), another guanosine-nucleotide-exchange factor for Ras GTPases, participates in T-cell signalling responses. Ras also initiates PI3K- and Akt-mediated signalling pathways in human peripheral blood T-lymphocytes [33]. Lymphocyte activation and proliferation induced by IL-2 are potently blocked by the Ras prenylation inhibitor L-778,123 [30]. A persistent impairment in the activation of Ras has been observed in peripheral blood mononuclear cells from patients with Type 1 diabetes [34].

There are only a few studies describing the effect of ACEi treatment on lymphocytes. Eiam-Ong et al. [35] reported that continuous ACEi treatment could abrogate AngII-stimulated circulating lymphocyte apoptosis induced by unilateral ureteral obstruction, and Oyama et al. [36] showed that ACEi treatment could reduce the level of GPCR (G-protein-coupled receptor) kinase in lymphocytes from patients with congestive heart failure. Our present results in lymphocytes from patients with hypertension without diabetes show that antihypertensive ACEi treatment induced a small decrease in Ras activation only in the first month of treatment, but increased phospho-ERK expression in the first week and also in the last 2 months of the study. In patients with hypertension and diabetes, ACEi treatment increased Ras activation only in the first week, but reduced phospho-ERK expression in the third month of treatment. It is important to emphasize the different effects induced by diuretic treatment in patients with hypertension without or with diabetes: the increase in Ras activation after 2 months disappeared in patients with hypertension and diabetes, and the reduction in phospho-ERK expression was not present after 2 months in these patients. It is also important to observe that, although there was an increased expression of Ras-GTP in patients with hypertension without diabetes, the levels of phospho-ERK expression were reduced. Masuelli and Cutler [44] reported that the increased expression of the Ras suppressor Rsu-1 enhances ERK2 activation in cultured fibroblasts, suggesting that Ras may have an inhibitory effect on ERK phosphorylation in some circumstances. ERK1/2 inhibits the activity of the Ras activator Sos (Son of sevenless), and Ras stimulates the PI3K/Akt pathway, which is known to inhibit Raf and, therefore, ERK [45]; however, in our experimental model, there were no changes in phospho-Akt activation after treatment with diuretics. Thus such a direct relationship between Ras-GTP and phospho-ERK is not clear.

Previous studies by Horiuchi et al. [46] were undertaken to investigate the potential effect of the cross-talk of the AT1 receptor and a HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase inhibitor (statin; also a Ras prenylation inhibitor) in VSMCs. Pre-treatment
of VSMCs with fluvastatin significantly inhibited AngII-mediated ERK activation; consistent with their in vitro results, phosphorylation of ERK was attenuated by the co-administration of valsartan and fluvastatin even at low doses in vivo, suggesting that the cholesterol-independent inhibition of AT1-receptor-mediated VSMC proliferation by statins may contribute to the beneficial effects of statins combined with ARBs in vascular diseases. Continuous treatment with ARBs could abrogate apoptosis induced by unilateral ureteral obstruction in circulating lymphocytes [35]. However, our present experiments have shown that ARB treatment did not appear to influence Ras activation and their main signalling pathways (MEK/ERK and PI3K/Akt) in human lymphocytes.

Our present findings support the view that in some patients and circumstances, such as the presence of diabetes, and depending on the hypertensive treatment, small GTP-binding proteins and MAPK may be promising therapeutic targets against cardiovascular diseases, as has been reviewed recently [47,48]. The design of new pharmacological strategies against these targets may be useful to diminish the damage induced by hypertension and diabetes.

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REFERENCES

4 Benter, I. F., Francis, I., Khan, L. et al. (2005) Signal transduction involving Ras-GTPase contributes to development of hypertension and end-organ damage in spontaneously hypertensive rats-treated with t-NAME. Pharmacol. Res. 52, 401–412
18 Rodríguez-Peña, A. B., Grande, M. T., Eleno, N. et al. (2008) Activation of ERK1/2 and Akt following unilateral ureteral obstruction. Kidney Int. 74, 196–209


